Volatile Components of Milk Fat Steam Distillates Identified by Gas Chromatography and Mass Spectrometry [1, 2]

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Abstract

Vacuum steam distillates of butteroil, fresh raw cream, fresh pasteurized cream, and pasteurized stored cream were analyzed by packed column and open-tubular column gas chromatography in conjunction with mass spectrometry. High-temperature (210 C) distillations of different butteroils yielded over 120 volatile compounds. Identification (or tentative identification) of more than 100 of these compounds was made from mass spectral–gas chromatographic data. Over 30 volatiles not previously reported in milk products were encountered. Many of the volatile compounds were obviously heat-produced, as the number of them was small in fresh, raw cream compared to heated cream and butteroil. However, aromatic compounds and some aliphatic hydrocarbons not previously reported were found in fresh, raw cream. Control experiments were conducted to determine laboratory contaminants and distillation artifacts.

The volatile compounds present in milk fat have been reported by several laboratories (13, 16, 17, 22, 32). Our work on identification of milk fat volatiles has been carried out in connection with efforts to define chemically the flavor of butter. Variation in the volatile composition of noncultured milk fat due to chemical changes is related to the stage and extent of oxidation of the milk fat (8, 10, 11, 12), the severity of heat treatment given the milk fat (21, 29, 31), the conditions and length of storage of the milk fat (25, 33), and the extent of browning reactions (30).

Mass spectrometry (MS), corroborated with gas-liquid chromatography (GLC), has been employed in our laboratories for several years as a means for identification of volatile compounds from dairy products (2, 5, 7, 21, 22). Although many of the compounds reported in this study have been reported in oxidized, irradiated, heated, and cultured dairy products, sometimes it is not clear if the specific treatment given the dairy product produced certain compounds reported, or if they were normal components of the untreated sample. In our experiments, the effects of heating cream and milk fat and of storage of cream and butter were studied; efforts were made to minimize oxidative changes and to classify separately compounds which were experimental artifacts. About 34 compounds not previously reported in milk were identified or tentatively identified.

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Experimental Methods

Steam distillations. Batches of cream and butteroil described in Table 1 were steam distilled at 1-4 mm Hg (pump end of system) in the apparatus shown in Fig. 1. The temperature of the cream during distillation averaged 20 to 30 C and was not allowed to exceed 40 C unless higher temperature effects were being studied. Approximately 20% of the cream volume was collected as aqueous distillate in about six hours.

Butter was made from fresh pasteurized cream, cooled in 50-60-lb. blocks, and refrigerated. Butteroil was prepared by cutting away the outer inch of the block, melting it at 40-50 C, and filtering the oil phase through Eaton and Dikeman no. 17 large-pore filter paper. Distillations of butteroil at temperatures above 100 C were conducted by initially heating the oil to the desired maximum temperature and maintaining this temperature for two hours, after which the heat was removed and distillation continued until the oil had cooled to 100 C. Three to four liters of aqueous distillate were collected from six to eight liters of butteroil.

Solvent extraction. Diethyl ether (Baker analyzed reagent grade), used to extract the dry ice–acetone/ethanol-cooled traps (Fig. 1 and Table 1), was purified by distillation over sodium metal. The aqueous distillate was made alkaline (pH 8) before extraction with ethyl ether, thus excluding free fatty acids from the

TABLE 1. Description of fractions of cream and butteroil subjected to vacuum steam distillation.

No.	Substrate distilled	Substrate storage conditions	Distillation temperature
1	Raw sweet cream	1 day at 3 C	20 ± 5 C
2	Pasteurized (71 C for 1.5 hr) sweet cream	7 days at 3 C	20 ± 5 C
3	Residue from Substrate 2	7 days at 3 C	20 ± 5 C
4	Butteroil made from pasteurized stored sweet cream butter	Butter stored 6 months at −18 C	100 ± 10 C
5	Pasteurized (71 C for 1.5 hr) sweet cream	8.5 months at −18 C	30 ± 5 C
6A	Raw sweet cream	1 day at 3 C	25 ± 10 C
6B	Raw sweet cream	7 days at −2 C	25 ± 10 C
7	Raw sweet cream	2 days at 3 C	30 ± 10 C
8A	Fresh butteroil (made from pasteurized sweet cream butter) containing 3% (v/v) butter serum	1 day at 3 C	140 ± 10 C
8B	Residue from 8A	7 days at 3 C	190 ± 10 C
9[a]	Fresh butteroil from pasteurized sweet cream butter	Butter stored 2-11 days at 3 C	210 ± 20 C

[a] Six separate butteroil samples were distilled under comparable conditions. In this table and Table 3, the six substrates are collectively no. 9.

volatile concentrate. Free fatty acids were noted in the fractions brought to pH 3 before extraction (Substrates 6A and 6B). The bulk of the ether was removed by slow distillation (22).

Packed column gas chromatography. A polar column (3.1 m by 0.32 cm od) was packed with 3.4 g of 20% Carbowax 20 M on acid-base washed Celite 545, 80-100 mesh. A nonpolar column (3.7 m by 0.32 cm od) was packed with 5 g of 20% Apiezon M on acid-base washed Celite 545, 80-100 mesh. Using suitable flow rates for 0.32-cm columns (22-28 ml/minute), several isothermal and temperature-programmed GLC analyses of the volatile fractions were conducted with both the polar and nonpolar columns to establish retention time (tentative) identifications of the milk fat volatiles. Chromatography was done with an F & M Model 810, an Aerograph 204, and a Barber-

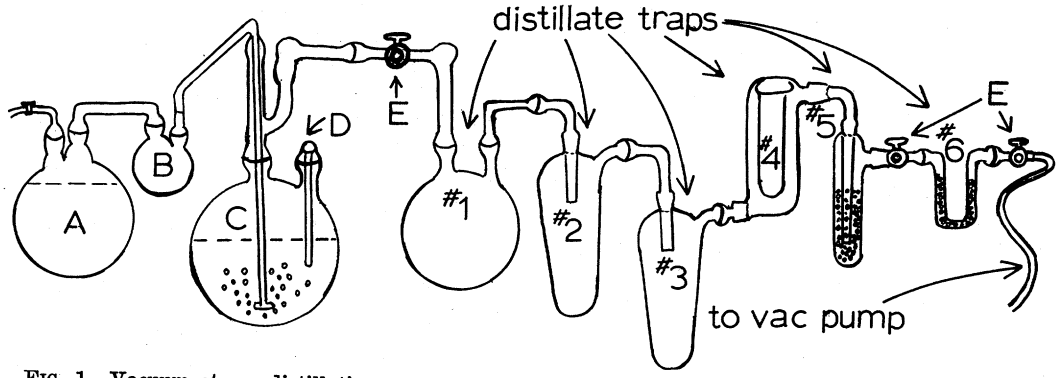

FIG. 1. Vacuum steam distillation apparatus used. A, steam reservoir; B, safety trap; C, substrate receptacle; D, thermometer well; E, stopcocks; Traps 1 and 2—dry ice–acetone cooled; Traps 3 through 6, liquid nitrogen–cooled traps (Traps 5 and 6 contain glass beads).

Colman Model 5000, all equipped with flame-ionization detectors. On-column injection was employed throughout all analyses. Odors of the peaks were noted by splitting the column effluent 3/1 or 1/1 (detector/atmosphere).

Open tubular column gas chromatography. Three 0.025-cm id open tubular (Golay) columns prepared by Perkin-Elmer Company were used to obtain more detailed analyses of the volatile fractions. The columns were a 91-m Apiezon L column, a 45-m Apiezon L column, and a 91-m Ucon (polar) column. The Barber-Colman Model 5000 was modified for open tubular column GLC by installing a removable injection splitter and a nitrogen gas make-up line to the flame detector. Column flow rate was set from 1 to 2 ml/minute by adjusting the splitter so that 60-99% of the carrier gas (N) did not enter the column. Isothermal and temperature-programmed runs were conducted at oven temperatures from 60 to 230 C (150 C maximum for the Ucon column). Splitter and detector temperatures were 210 and 230 C, respectively. The volatile concentrates were enriched with known compounds in repeated GLC runs on the Ucon column. Simultaneous emergence of unknown peaks and known compounds established GLC identifications.

Preparative gas chromatography. The 0.32-cm Apiezon M column placed in an Aerograph 90-P3 gas chromatograph was used as a preparative GLC column to obtain from the entire distillate concentrate more narrow boiling ranges of volatile compounds. Up to 20 μliters of sample were injected into this column, of which about 15 μliters were recovered. Bands of the entire volatile spectrum containing 5 to 15 peaks each were collected separately in 25-cm (length) glass melting point capillary tubes (1-mm id), which were cooled with dry ice or dry ice wetted with ethanol. After trapping the separate bands, capillaries were sealed and kept at −10 C. Samples trapped in this manner were not contaminated with water.

Control experiments. Chloroform was a major volatile in Substrates 1 through 5 (Table 1). To exclude contamination of fractions by laboratory solvents (chloroform, ethanol, benzene, hexane, etc.), Substrates 6 through 9 were distilled in a laboratory from which all liquid organics except ethyl ether and ethanol were removed. Under these conditions, chloroform was present in only very minor amounts.

The distillate in Trap 1 (Fig. 1), assayed separately in butteroil distillates by preparative thin-layer chromatography, followed by infrared spectrometry (Beckman IR-5A), demonstrated that cholesterol, diglycerides, and even triglycerides were removed from the oil during distillation above 200 C. The efficiency of recovery from high-temperature distillations was estimated to be greater than 60% for δ-tetradecalactone and approached 100% for the more volatile compounds (up to 2-tridecanone).

One sample of butteroil was distilled initially at 45 C, then redistilled at 230, then 210, 120, and finally at 235 C, collecting the volatiles separately after each distillation. The fourth distillation (120 C), which yielded only traces of the usual volatiles, provided the experimental blank distillation. Distillation of the 120 C-residue at 235 C produced several volatile compounds whose presence was attributed solely to high-temperature treatment of the oil. Peroxide values (meq of peroxide/kilogram fat) were measured on several fresh fat and stored fat samples. Values ranged from 0 to 0.8, showing a low degree of autoxidation in our samples.

Combined mass spectrometry and gas chromatography. An F & M Model 810 gas chromatograph was used for all mass spectral work. Analyses of volatiles of Substrates 1 through 5 (Table 1) were carried out by connecting the Carbowax 20 M column to an Atlas CH-4 mass spectrometer (21). The column effluent from the gas chromatograph was split with part (10%) of the effluent stream passed to the flame detector and the remaining part routed to the EC-1 gas inlet system. A portion (up to 5%) of the effluent stream routed to the EC-1 inlet was bled into the mass spectrometer. Temperature programming was used for packed column-MS analyses, since the mixture injected included low-boiling to very high-boiling (>200 C) compounds. Operating parameters for the mass spectrometer were essentially those used by Arnold et al. (2), except that a 70-ev ion source was used for fragmentation and a separate 20-ev source provided a GLC record. The filament currents of the two ion sources were 60 and 30 μamp for the 70- and 20-ev sources, respectively. The mass spectrometer analyzer pressure was 10^{-6} mm Hg.

To prevent thermal breakdown of certain compounds, the line carrying the GLC effluent to the mass spectrometer was held at about 210 C. Conversion of *n*-hexanal to benzene occurs at temperatures above 220 C (19).

Fractions 7 through 9 (Table 1) were analyzed by means of the 91-m by 0.025-cm Apiezon L column, described above, connected to the Atlas CH-4. The total effluent from the open tubular column was fed into the high vacuum of the mass spectrometer double ion

source, providing a simultaneous GLC record (20 ev, 35 μamp filament current) and a fragmentation pattern (70 ev, 8 μamp filament current).

For open-tubular column-MS analyses, isothermal runs of 60, 100, 130, 150, 170, and 215 C were employed, depending on the boiling point range of the sample injected. From 0.2 to 2 μliters of sample (obtained by preparative GLC as described previously) were injected at the front of the column; the injection port split ratio was 99/1.

Results

In this study, a compound was considered identified when its GLC peak yielded mass spectra which best fit that of a single known compound, and GLC retention data confirmed the mass spectral identification. Retention times on several columns and checks on retention times by the sample enrichment technique (see Methods) on the Ucon column supported our identifications. A typical Ucon column analysis is shown in Fig. 2 and Table 2. Tentative identification is proposed when MS or GLC evidence is incomplete but is sufficient to suggest that the unknown is a specific compound.

Mass spectral data obtained for aromatic positional isomers such as dimethylbenzene were not sufficiently accurate to determine if the compounds were ortho, meta, or para. The mass spectra of dimethylbenzene, methylethylbenzene, and dichlorobenzene were observed in separate GLC peaks, demonstrating the presence of more than one isomer. Evidence for more than one dimethylbenzene, one dichlorobenzene, and one methylethylbenzene was also obtained on the open-tubular columns (e.g., Fig. 2, Peaks 32, 33, and 36).

Compounds identified in the different substrates (Table 1) are given in Table 3. Compounds in Table 3 do not include those found in every sample and also repeatedly by other investigators. All substrates studied here contained the odd C-number methyl ketones from C_3 through C_{15}, *n*-hexanal, butanone, ethanol, ethyl formate, ethyl acetate, chloroform, δ-C_8, C_{10}, and C_{12} lactones, ethanal, benzene, and toluene. The causes of variation in volatile composition of the fractions, as observed in Table 3, are considered in the Discussion.

Mass spectral comparisons of known compounds to unknowns are given in Table 4 for several of the previously unreported compounds. Mass/charge peaks significant in identification of the compound are recorded. In most cases, more than seven peaks were correlated with the known in making identifications.

TABLE 2. Identification of numbered peaks in Fig. 2.

No.	Compound
1	Unknown
2	Acetaldehyde
3	Diethylether
4	Propional
5	Acetone
6	1-Hexene
7	Ethylformate
8	Unknown
9	*n*-Butanal
10	Butanone
11	Heptane
12	Ethanol + ethylacetate
13	1-Heptene
14	Unknown[a]
15	Benzene
16	Unknown[a]
17	2-Pentanone
18	*n*-Pentanal
19	*n*-Octane
20	1-Octene
21-22	Unknowns
23	Toluene
24	Unknown[a]
25	Ethylbutyrate
26	2-Hexanone
27	*n*-Hexanal
28	*n*-Nonane
29	1-Nonene
30	Unknown
31	Ethylbenzene
32	*p*-Xylene
33	*m*-Xylene + unknown
34	2-Hexenal (?)
35	2-Heptanone
36	*o*-Xylene + *n*-heptanal
37	1-Decene
38	Styrene
39-42	Unknowns
43	Ethylhexanoate
44	Unknown
45	2-Octanone
46	*i*-Butylbenzene (?)
47	*n*-Octanal
48	*n*-Undecane
49	1-Undecene
50	Benzaldehyde
51	Unknown
52	*o*-Dichlorobenzene
53	2-Nonanone
54	*n*-Nonanal
55	*n*-Dodecane
56	1-Dodecene

[a] Present only in high-temperature distillates.

Discussion

The number of volatile compounds and their amounts in fresh, raw, unheated cow's milk and unheated butteroil is small in comparison to the number of volatiles when the milk is heated, irradiated, stored, or becomes oxidized (12, 13, 17, 23, 29, 30, 31, 32, 33). A number of excellent experiments has been conducted to determine specific causes and specific precursors of certain volatiles (9, 10, 16, 27, 28).

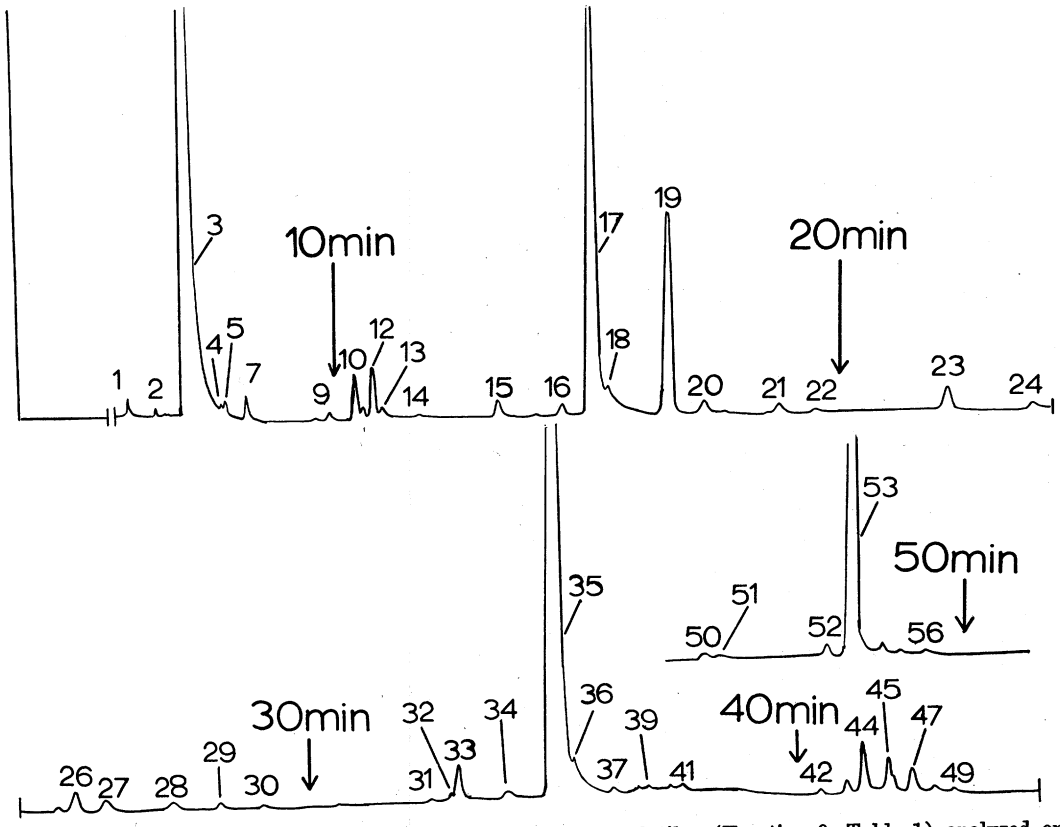

FIG. 2. Gas chromatograms of butteroil steam distillate volatiles (Fraction 9, Table 1) analyzed on the Ucon open-tubular column (see text); isothermal at 50 C for 15 min, 7.5 C/minute to 150 C. Some 25 peaks beyond 1-dodecene (no. 56) are not shown. The numbered peaks are identified in Table 2.

The present work was not intended to determine the nature of specific precursors of volatile substances reported in the steam distillates. However, since certain compounds appeared only in substrates treated in a specific manner (Table 3), a tentative assessment can be made as to the nature of the chemical reactions producing certain classes of volatiles. Furthermore, control experiments were conducted to determine which compounds were experimental artifacts, as opposed to those originating in milk fat.

Autoxidation products. Normal hexanal was present between 0.1 and 1 ppm (estimated from GLC peak size) in all samples of cream and milk fat. Low levels of normal pentanal, *n*-heptanal, *n*-octanal, *n*-nonanal, ***n*-undecanal,** 2-hexenal, and 2-heptenal were also sporadically noted in some fractions. The C_5, C_6, and C_7 normal alkanals are major components of autoxidized milk fat (8, 10, 12). Low peroxide numbers (<0.8 meq/kilogram) and relatively low amounts of normal alkanals show that autoxidation was measurable but not extensive in our fractions.

Bacterial end products. The time at room temperature and storage of Substrates 2, 3, and 5 (Table 1) would allow such organisms as Pseudomonas to reduce aldehydes to the corresponding alcohols (18). Thus, Substrate 3 contained, uniquely, in our fractions 1-propanol, 1-butanol, and 1-hexanol. Bacterial activity in Substrate 5 was also suspected, as 2- or 3-methylbutanal (24) and relatively high levels of diacetyl and ethanol (4) were noted during GLC assays by retention times and odors of emerging peaks.

Heat effects. Odd C-number methyl ketones, lactones, dimethyl sulfide, butanone, and hydrogen sulfide increased during the pasteurization of cream. Our work shows that heating butteroil produces also 2-hexanone, 2-octanone, 2-decanone, 2-dodecanone, and probably 2-tetradecanone. Peak areas of even-numbered methyl ketones were 1-3% of odd-numbered methyl ketones, and their rate of increase with

TABLE 3. Identified volatiles of steam distillates of fresh cream, heated cream, heated and stored cream, and heated butteroil. (*Continued*)

Compound identified by MS-GLC analyses	Experimental fractions containing compound[a]								
	1	2	3	4	5	7	8A	8B	9
2-Hexanone							+	+	+
2-Octanone							+	+	+
2-Decanone							+	+	+
2-Dodecanone								+	+
2-Tetradecanone									+
3-Heptanone	+			+					
n-Propional	+	±	+	±	+		±		+
n-Butanal	+	+	+	+			±		
n-Pentanal	−		−	+					+
n-Heptanal	±		+	+	+		+	+	+
n-Octanal	±		±				+	+	+
n-Nonanal	±			+	+		+	+	+
n-Undecanal				±					+
2-Hexenal	−			±					+
2-Octenal									±
3-Methylbutanal	−			−	±				−
1-Propanol	−		+	−	−	−	−	−	−
1-Butanol	−		+			−	−	−	−
1-Hexanol		−	+						
2-Butanol	+	−					−		
Methylformate							−		±
Methylacetate	−	±	±	+	+		−		
Methyldecanoate									+
Ethylbutyrate	−					−		+	+
Ethylhexanoate	−		+	±		−	+		+
Ethylheptanoate									+
Ethyloctanoate	−					−	+		+
Ethyldecanoate	−			±				+	+
Ethyldodecanoate	−								±
Diacetyl		+	+		+	±	+		
Diethoxymethane	−		+	+	+		+		+
1,1-Diethoxyethane	−	−		+	+	+	+		+
1,1-Diethoxypentane								+	
1,1-Diethoxypropane				+	+				+
Triethylorthoformate					±				
Ethylene-diacetate			±		+	−	+	±	−
2-Furfural	−			−			+	+	
5-Methyl-2-furfural							+		+
Dimethyl sulfide	+	+	+				+		+
δ-Hexalactone							±		−
δ-Heptalactone								+	±
δ-Nonalactone								+	+
δ-Tetradecalactone								+	±
δ-Hexadecalactone									±
Dimethylbenzene[b]	+	+	+	+	+	±	+	+	+
Styrene			+	±					+
Methylethylbenzene[b]	+	±	+	±	+	±	+		+
n-Propylbenzene			+						±
Isopropenylbenzene	+			+			±		
A Methyl-n-propylbenzene			±						
Isopropylbenzene	±								
A Trimethylbenzene				+			+		±
Ethylbenzene	+	±	±	+			+		+
A Diethylbenzene					±				
Phenyl-C_4-alkane[c]		±	+		+		+	+	+
1-Phenylhexane								+	
1-Phenylheptane								+	
Benzaldehyde	−	−	+				±		+
A Dihydroxytoluene									+
1-Phenylpropane-dione							±		±
A Butyldimethoxybenzene								±	
A *t*-Butyhydroxyanisole								+	
1,1-2,2-Tetrachloroethane				+					
A Dichlorobenzene		+	+	+			+		+
A Chlorotoluene		+					+		

TABLE 3. Identified volatiles of steam distillates of fresh cream, heated cream, heated and stored cream, and heated butteroil. (*Concluded*)

Compound identified by MS-GLC analyses	Experimental fractions containing compound[a]								
	1	2	3	4	5	7	8A	8B	9
n-Hexane	−			−		+	+	±	+
n-Heptane	−	−	±	−		+	+	+	+
n-Octane	−	−	−	+		+	+	+	+
n-Nonane	−			−				+	+
n-Decane	±	−		±			+	+	+
n-Undecane				−			+		+
n-Dodecane	−	−	−	±					+
n-Tridecane				−					+
n-Tetradecane		−		−			+		±
n-Pentadecane									+
n-Hexadecane									+
n-Heptadecane				+					
2,2,3,3,5,6,6-Hepta-methylheptane	±	−		−		+			±
1-Heptene		±			−		±	+	+
1-Octene						±			
4-Octene		−		±			+	+	+
1-Nonene	+	±	+	±	+		+		+
1-Decene	±	−		−			+	+	+
1-Undecene				−			+	+	+
1-Dodecene		−		−			±		±
1-Tridecene		−		−				+	+
1-Tetradecene									±
1-Hexadecene							+		±
1-Heptadecene									
α-Pinene	±			±					
Pinane									
Myrcene	+								
2-Methylpentene			±						

[a] Experimental substrates are described in Table 1; the numbers here refer to the substrate numbers in Table 1. Symbols: (+), positive MS identification and confirmation of identification on more than one GLC column; (±), tentative identification; and (−), complete absence of a GLC peak for the compound. Blank spaces mean no MS data for the compound was noted, but its presence could not be ruled out from GLC data. Compounds repeatedly detected in milk fat are not included (e.g., odd-number methyl ketones, normal alkanals, and certain lactones). The composite results from six comparable fractions are recorded under Fraction 9. Acetals and ethylheptanoate were found in only one of the six fractions. Aromatics above 8 C-atoms were sporadically noted.

[b] At least two, and probably three, xylenes were noted on GLC chromatograms and in mass spectra. Evidence was also found for two or three isomers of methylethylbenzene and dichlorobenzene.

[c] The type of side chain (*n, iso, sec*) could not be ascertained from MS data.

heat paralleled the odd-number methyl ketones. Ethyl esters of butanoate, hexanoate, octanoate, decanoate, and dodecanoate were more prominent in heated milk or in heated butteroil which contained some butter serum (buttermilk). In addition to 2-furfural (2), 5-methyl-2-furfural was detected when cream or butteroil containing some butter serum (nonlipid phase of butter) was heated above 140 C. This substance is a known browning reaction product (15, p. 472), but has not been reported in milk. Vanillin, hydroxymethylfurfural, maltol, and methyl propanal, all previously noted in heated milk (26, p. 308), were not detected.

One sample of cream which definitely had been scorched during a steam distillation (caused by cream solids baking on the container), contained dimethyl disulfide, a di-*t*-butylhydroxytoluene, and 1-phenylpentane. These substances are not normally found in raw milk or even in pasteurized cream.

Diethylacetals were present in substrates which were either aged or in butteroil substrates distilled in the presence of butter serum (Table 3). We did not detect acetals in fresh butteroil or fresh cream. Ethanol increases drastically in aged or decomposed cream (32) and the presence of acetals seemed to be proportional to the ethanol content of the volatile mixture.

Many of the same alkanes and 1-alkenes detected in our fractions have been noted in oxidized butteroil (12), in irradiated butteroil (19, 23), and in oxidized vegetable oil (20). Although occasional aliphatic hydrocarbons were detected in unheated cream, there was a definite increase in alkanes and alkenes when butteroil was heated above 200 C. After removal of all volatiles from a butteroil sample

TABLE 4. Comparison of mass spectra of milk fat volatiles with mass spectra of known compounds.

	m/e Intensities of given peak						
m/e Peak[a]	91	106	105	51	77	78	63
o-Dimethylbenzene[b]	100	57	24	16	13	7	6
Unknown[c]	100	55	23	11	18	9	9
m/e Peak	105	120	77	91	106	65	63
p-Methylethylbenzene[b]	100	31	8	8	9	4	3
Unknown[c]	100	25	8	9	10	5	5
m/e Peak	105	120	119	39	77	27	.....
m-Trimethylbenzene[b]	100	67	16	15	13	10	.....
Unknown[c]	100	50	20	25	12	20	.....
m/e Peak	91	92	65	105	51	78	135
n-Butylbenzene[b]	100	55	11	9	7	6	3
Unknown[c]	100	50	11	10	5	6	2
m/e Peak	105	77	51	106	50	78	148
1-Phenylpropane-dione[d]	100	62	15	8	5	5	3
Unknown	100	61	28	11	11	6	5
m/e Peak	58	43	58	71	57	41	156
2-Decanone[d]	100	60	33	29	15	10	10
Unknown	100	90	25	30	16	20	4
m/e Peak	57	72	85	114	55	58	56
3-Heptanone[b]	100	18	24	8	4	4	3
Unknown	100	19	18	9	7	2	6
m/e Peak	59	47	87	103	75	27	41
1,1-Diethoxypropane[b]	100	64	61	43	33	31	16
Unknown	100	66	56	33	39	55	33
m/e Peak	41	93	69	53	67	94	121
Myrcene[d]	100	81	66	19	15	10	10
Unknown	100	64	48	29	22	14	12
m/e Peak	41	55	56	70	42	83	112
trans-4-Octene[b]	100	99	44	40	30	24	25
Unknown	100	100	50	45	44	25	20
m/e Peak	110	109	53	27	39	51	38
5-Methyl-2-furfural[e]	100	89	77	48	21	15	11
Unknown	100	96	88	60	30	30	12

[a] Up to seven significant mass/charge peaks are shown here. More peaks were correlated in the actual identification.
[b] Reference (1).
[c] Spectra not sufficiently accurate to determine which positional isomer was present.
[d] Reference (3).
[e] Reference (6).

by three successive distillations (45, 230, and 210 C), this oil was distilled at 120 C. Only traces of high-boiling volatiles were noted and no alkanes or alkenes. The oil was again distilled, this time at 235 C. A large peak containing *n*-octane was noted. Smaller peaks contained C_6, C_7, and C_9 to C_{17} normal alkanes. Still smaller peaks of 1-alkenes from C_7 to C_{18} chain lengths were observed. Temperatures of 235 C are not sufficient to crack long-chain hydrocarbons to any measurable extent.[3] The other compounds (aldehydes) noted in irradiated and oxidized samples (12, 19, 23) were not quantitatively and qualitatively the same as we observed. Therefore, it is not probable that chain fission or oxidation are the mechanisms of hydrocarbon formation here, although heat definitely promoted their appearance in milk fat.

Aromatic compounds and terpenes. A growing number of workers are reporting aromatic compounds in food volatile mixtures (7, 13, 14, 19, 20, 21, 23). Aromatics other than benzene, toluene, xylenes, and methylethylbenzenes were found sporadically and are probably derived from cattle feed. Oxygen-containing benzene derivatives may have been produced by heat; however, certain phenols have been reported in fresh butteroil (13) which was not highly heated. Butylated-hydroxytoluene has been

[3] Bond dissociation energies of carbon-carbon bonds and kinetic approximations support this statement. Cram and Hammonds' Organic Chemistry (McGraw-Hill, 1959) on page 604 gives 450 C as the temperature at which carbon-carbon single bonds begin to break.

found (unpublished data) in the laboratory-distilled water supply and may well account for the antioxidants in some fractions.

Myrcene and other terpenes and miscellaneous substances such as heptamethylheptane and tetrachloroethane are thought to arise from the feed source, as they are not known bovine metabolites. Terpenes have been noted in dairy products by other workers (7, 21).

The present work, and previous literature on milk volatiles, would suggest that the number of new compounds that will periodically be reported in milk will diminish, at least until smaller amounts of organic material can be detected and identified. The present work adds to the list of authentic milk fat volatiles that may be in fresh milk or may arise during normal processing procedures. These would include 2-decanone, 2-dodecanone, 2-tetradecanone, a variety of aromatics, some terpenes, and traces of hydrocarbons. Miscellaneous substances noted sporadically may arise from the cattle feed. A few of the compounds reported here may be post-milking contaminants (e.g., antioxidants) or arise only from abnormally high temperature treatment.

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